



C-Toaster using

Goal : How to use the Cells-Toaster plug_in

Note

The Cells-Toaster plug-in is composed from the two distinct tools: the **Cell Analyzer (Celer)** and the **Cell Files Analyzer (Cefiler)** *actually in development*. It proposes **batch image processings**.

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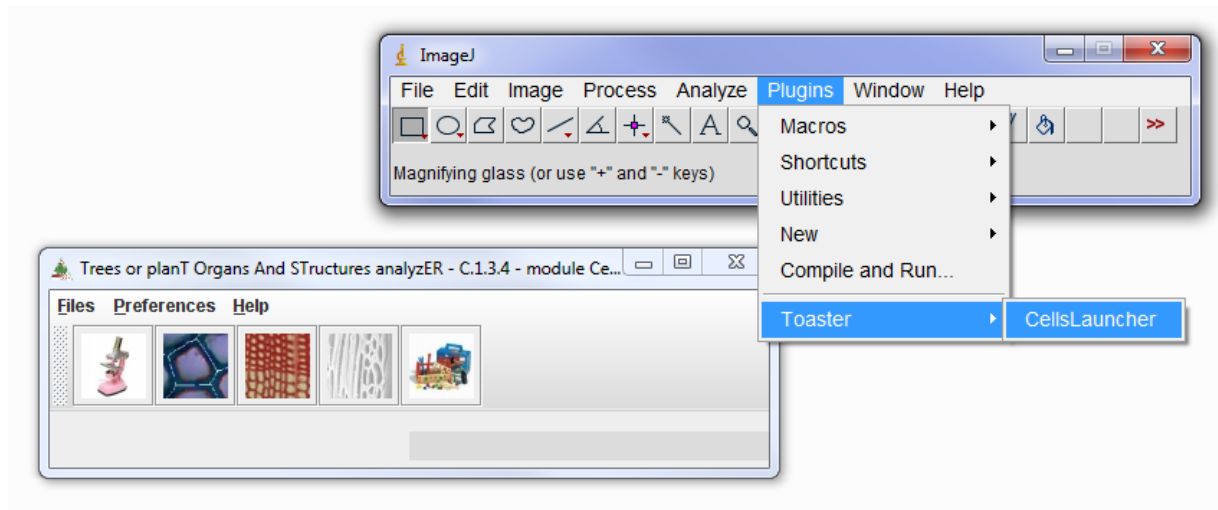
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1. Toaster running

The *Toaster* plug-in is run from the *ImageJ*¹ application. So,

- Run ImageJ...
- Select the *launcher* item of the *Toaster* sub menu of the *plugins* menu



Im. 1 : ImageJ et le plugin C-Toaster (Cells)

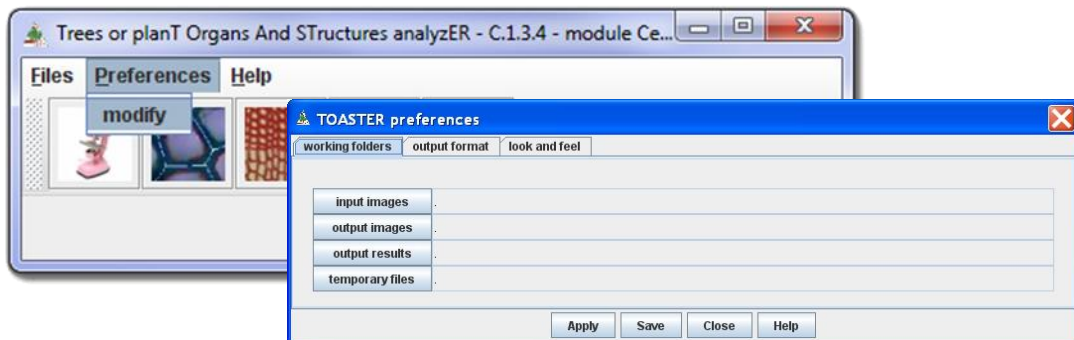
¹ **ImageJ** is an open-source software developed by the *National Center for Biotechnology Information* (NCBI), for the images processing and analysis. It is distributed under GPL licence.



2. Toaster setting

The *Toaster* plug-in manages the working folders and the export format: the user can specify where find input data or achieve output results,... and this once and for all.

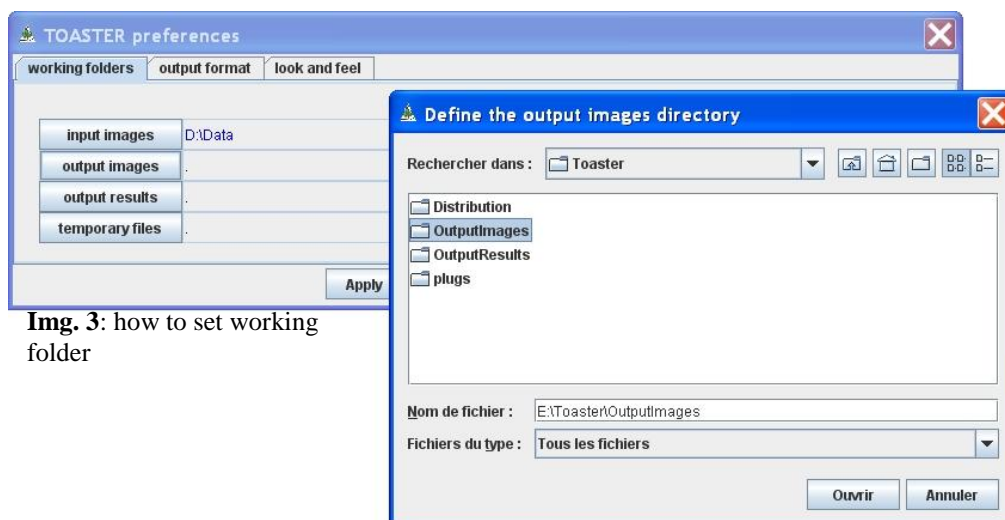
- Select the *modify* item of the *Preferences* menu



Img. 2: the folders setting

2.1. The working folders

In the *working folders* page, set the different fields from the corresponding browsers (*the buttons located at the left of the text fields*) (see img 3.). *Save* the settings if you want the application uses it at each time; in other case, *Apply* only the settings (see img 2.).



Img. 3: how to set working folder



Note: rules of colors (fig 4.)

Different colors are used to indicate the specified paths.

- ✓ In black: validate path already used – *the indicated folder exists and has been already used*
- ✓ In blue: path modified by the operator– *the indicated folder exists when it is selected from the browser*
- ✓ In red: invalidated path – *the indicated folder does not exist on the current computer; you should give a good path.*



Img. 4 : rules and colors to specify the working folders

The fields :

input images : folder in which the plug-in takes the images or the repertories containing the images to process.

output images : folder where the plug-in saves the results images, i.e. the visual outings used to check the processing.

output results : folder where the plug-in saves the results files, i.e. the numerical outings produced by the processing.

temporary files : folder where are achieved the temporary files.

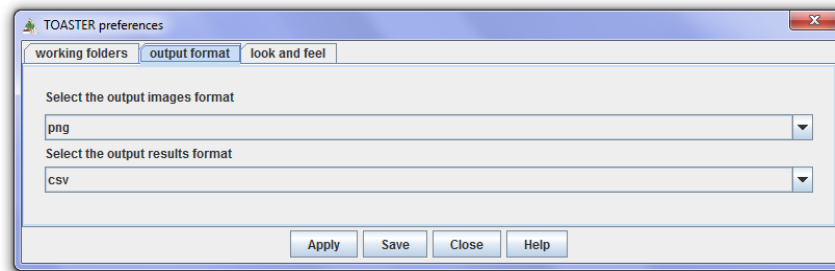
Note: folder syntax (fig 4.)

The working folders are normally given by their extend name, that is to say from the root. However, a relative path can be displayed. The “.” means the local ImageJ folder, that is to say the repertory where is installed the ImageJ application.



2.2. The export formats

In the page *output format*, set the different fields using the drop-down list (*voir img 5.*). **Save** the settings if you want the application uses it at each time; in other case, **Apply** only the settings (see *Img. 5*).



Img. 5 : format des fichiers d'export

The fields :

Images format: concerns the format of graphical results. Several graphical formats are available: bmp (*BitMaP*), gif (*Graphics Interface Format*), jpeg (*Joint Photographic Experts Group*), png (*Portable Network Graphics*) and tiff (*Tagged Image File Format*).

Results file format: concerns the format of numerical results. Several standard formats are available: txt (*TeXT*), csv (*Comma Separated Values*, loadable by the usual spreadsheets).

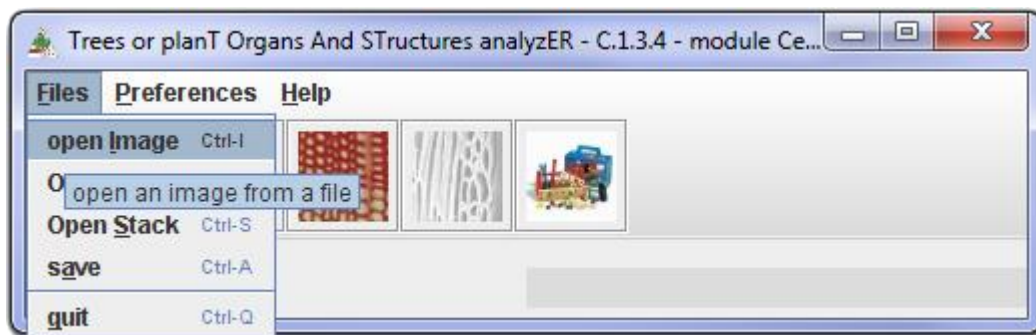


3. Data loading

Different modes are available: load (and process) only one file, or load (and process) all the files of a folder.

3.1. I want process only one file

Use the first submenu of the files menu... A browser is then opened and allows selecting THE file to process.

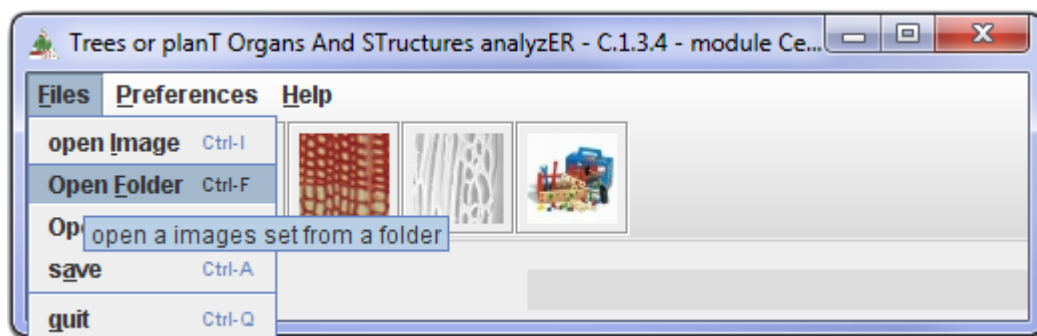


Note: results

The output data (image file or/and results file) will be saved into the output folders under the same root name as the selected file.

3.2. I want process several files

Use the second submenu of the files menu to select several files. A browser is then opened and allows selecting A file of the folder to process. *All the files of the folder will be processed.*



Note: results

The output data (image file or/and results file) will be saved into under the same root name as the selected file, achieved in a subfolder created in the output folders.



Warning: the input folder has to contain only image files produced from the same settings, with the same optical resolution (value generally given in dpi).

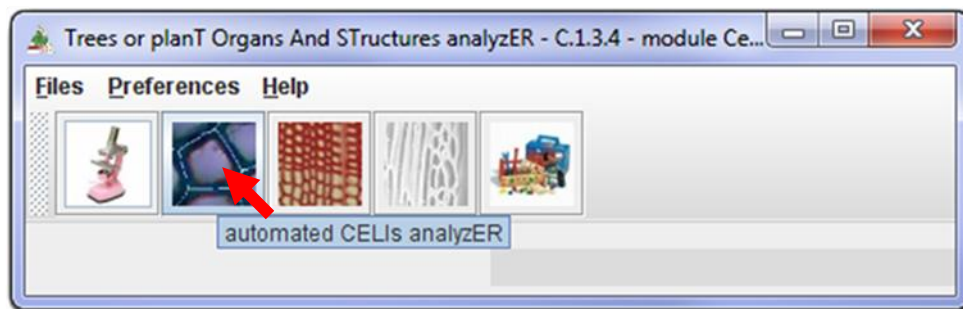
4. Cells analyzer (Celer) – 1.0.a

Celer is a half-automated plug-in used to analyze the geometry of cells (lumen size, wall size and thickness,...) provided by light microscopic images. The study processes only the sharp parts of the image: these parts are automatically detected by the application. The numerical results are saved in csv files, easily readable on spreadsheet.

Note

- ✓ The cell sharpness is evaluated by the positive difference between the mean lumen density and the mean wall density.
- ✓ The cell clustering, i.e. the distribution of cell points between lumen and wall is automatically done by k-means.

The Celer plug-in is opened from the first icon in the tools bar of Toaster

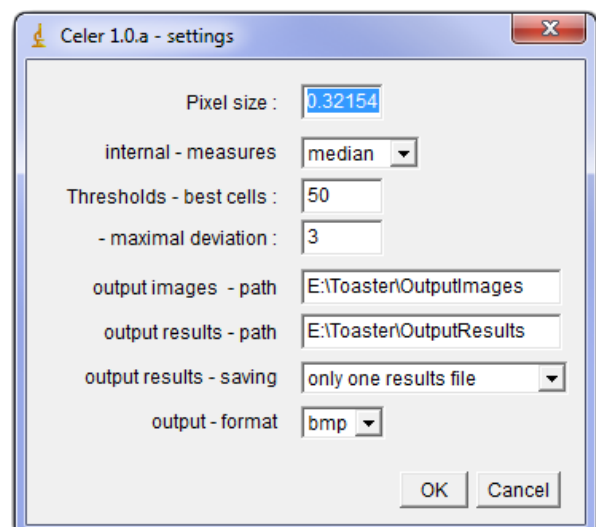


Note

- At least one image must be loaded before to open Celer plug-in .

4.1. Celer settings

The following dialog box allows adapting the processing to the current (set of) image(s). Three aspects are concerned: the metrical aspect to define the significant object sizes, the processing aspect and the managing aspect giving the working folders and the export formats.





The metrical aspect

Input – pixel size: this is the scale factor, i.e. the size in millimeter (or micrometer) of the pixel height (or width). This value is by example given subdividing 25,4 (the millimeter value of the inch) by the optical resolution of the image, *given in dpi (dot per inch). The dpi is set for the digitalizing step, or read from an images editor such as Gimp².*

The processing aspects

Internal - measures: defines which value (*see §8*) will be used to define the representative wall thickness. Default value is median thickness to not take account of outliers. The user can choose between different values: mean, minimal, maximal or median value, or the set of thicknesses evaluated from the wall border.

Note:

- ✓ The thicknesses are defined by a distance map between the two sides of the cell wall.
- ✓ If the *all* choice is selected, all the values are edited in the results file.

Threshold – best cells: gives the number of cells which will be considered in the results file.

Threshold – maximal deviation: the maximal value of the standard deviation of the thickness distribution of the cell wall. *This is the threshold for acceptability.*

The managing aspects

The managing aspects are set from the Preferences panel of the Toaster plug-in (see §2). Are editable the following fields:

Output images - path: extend name of the folder where will be achieved the output images.

This field is set from the Toaster settings. These images are built to avoid a visual checking after data treatment. The leaves are colored in regard to their position in the treated image: the hue depends on the line, and the saturation on the row in the line (§7).

Output results - path: extend name of the folder where will be achieved the output results.

This field is set from the Toaster settings. These files contain the numerical values of calculated parameters, sorted by natural reading order of leaves – from left to right, from up to down.

² The GIMP is a freeware to create and manage images and numerical photography.



Output results - saving: the way to archive the results

- ✓ **Only one results file:** the results of all read input files are written in only one output file.

The output file takes the name of the input folder (when several files are processed) or of the input file (when only one image is treated). In the results file, each line corresponds to one leaf; its name is defined by the following way:

[image_name] , [number of line]_[row of leaf into the line].

- ✓ **One results file per image:** the results are saved image per image, in different files.

The output files follow the same organization as the input file. In each results file, each line corresponds to one leaf of the corresponding image; its name, called identifiant, is defined by the following way:

[number of line]_[row of leaf into the line]

Output images format: give the format of the output images

Output results format: give the format of the output numerical files

The csv files are loadable by the usual spreadsheet; the txt files can be imported in the usual spreadsheet.

4.2. Celer running

The Celer plug-in is ran when you validate your settings: click on button “ok”.

4.3. Example

Data and results

The input data are three jpeg images located into the folder A317



NB: here, the images are produced with the same magnification.



The settings of the §4.1 give the following output data:

- ✓ the checking images achieved in the folder **E:\\Toaster\\OutputImages\\A317**



- ✓ One results file, *named A317.txt*, achieved in the folder **E:\\Toaster\\OutputResults**

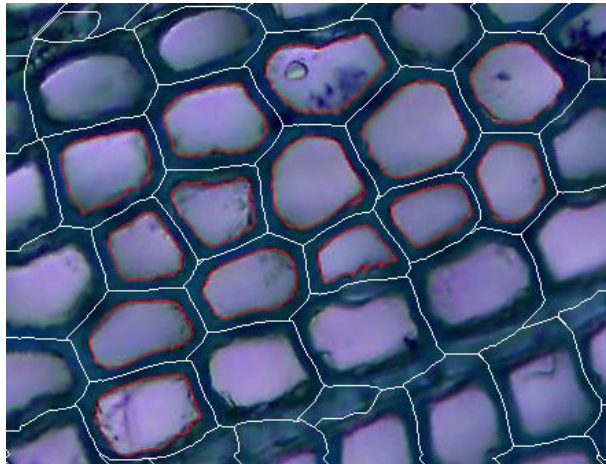
	A	B	C	D	E	F	G
1	Image	Sharpness	cell Area	Wall Area	Lumen Area	Thickness (med)	
2	test1	155.0976124	378.7101399	153.5311378	225.1790021	2.461984192	
3	test1	154.1807181	618.0532942	226.7298217	391.3234725	2.728356288	
4	test1	152.2510463	560.1560301	219.3892757	340.7667543	2.838692096	
5	test1	149.6493848	537.9276162	239.7567061	298.1709100	3.160232096	
6	test1	145.5419178	374.2644571	173.3816283	200.8828288	2.89386	
7	test1	143.4813602	466.0729759	206.4657792	259.6071966	2.89386	
8	test1	140.6434420	559.0187624	241.6176896	317.4010728	2.971878144	
9	test1	138.9720433	335.8041317	146.2939798	189.5101519	2.461984192	
10	test1	136.5876530	485.8200785	217.6316802	268.1883983	3.027046048	
11	test1	136.0074624	541.5461952	206.8793311	334.6668640	2.705506048	
12	test1	134.0443947	521.0753768	227.3501495	293.7252273	2.89386	
13	test1	132.8683784	406.2113404	184.6509172	221.5604231	2.595170240	
14	test1	129.5727200	308.1995433	158.9073123	149.2922309	2.57232	
15	test1	128.0000000	0.0000000	0.0000000	0.0000000	0.0000000	

Here, the results of different images are saved in the same results file. It is the user choice (§ 4.1)

The results are sorted for each image by increasing **cell sharpness** (§4.). Here, the thickness is represented by the median value. The areas are given in squared units (millimeters or micrometers according to the user settings)



Focus on visual results



The cell boundaries are displayed in white, the lumen boundaries in red. The wall is defined as the space between the white and the red curves.

This checking image allows to verify the accuracy of the image segmentation.

Focus on numerical results

	A	B	C	D	E	F	G	H	I	J	K	L
1	Sharpness	cell Area	Wall Area	Lumen Area	Thickness measurements....							
2	155.0976124	378.7101399	153.5311378	225.1790021	1.6077	1.685718144	1.740886048	1.740886048	1.740886048	1.740886048	1.818904192	1.818904192
3	154.1807181	618.0532942	226.7298217	391.3234725	1.6077	1.6077	1.6077	1.6077	1.685718144	1.685718144	1.740886048	1.740886048
4	152.2510463	560.1560301	219.3892757	340.7667543	1.818904192	1.92924	2.007258144	2.062426048	2.062426048	2.140444192	2.140444192	2.140444192
5	149.6493848	537.9276162	239.7567061	298.1709100	1.92924	1.92924	1.92924	1.92924	1.92924	1.92924	2.062426048	2.062426048
6	145.5419178	374.2644571	173.3816283	200.8828288	1.818904192	2.007258144	2.007258144	2.140444192	2.140444192	2.140444192	2.140444192	2.140444192
7	143.4813602	466.0729759	206.4657792	259.6071966	2.062426048	2.062426048	2.062426048	2.140444192	2.140444192	2.140444192	2.195612096	2.195612096
8	140.6434420	559.0187624	241.6176896	317.4010728	2.383966048	2.383966048	2.461984192	2.461984192	2.517152096	2.517152096	2.517152096	2.517152096
9	138.9720433	335.8041317	146.2939798	189.5101519	1.552532096	1.552532096	1.552532096	1.6077	1.6077	1.6077	1.685718144	1.685718144
10	136.5876530	485.8200785	217.6316802	268.1883983	1.874072096	1.92924	1.92924	1.92924	1.92924	2.007258144	2.007258144	2.062426048
11	136.0074624	541.5461952	206.8793311	334.6668640	1.552532096	1.6077	1.6077	1.6077	1.6077	1.6077	1.740886048	1.740886048

Here, all the thicknesses are given for each “good” cell of the image. These values allow studying the thicknesses distribution for each cell wall.

4.4. Reference

To come



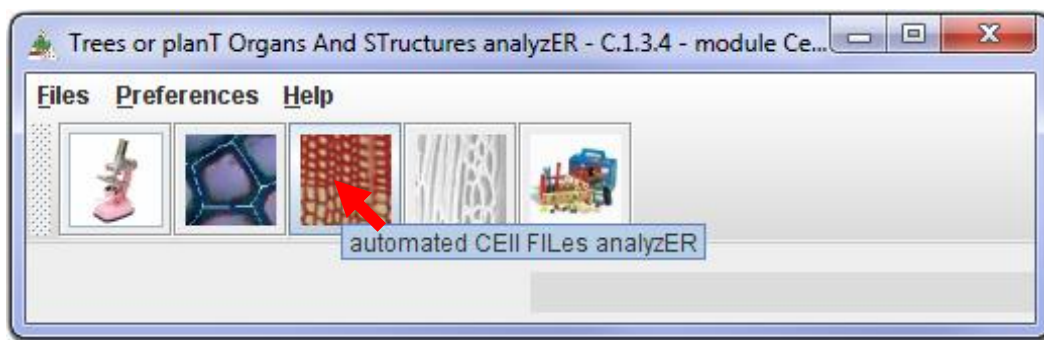
5. Cell files analyzer (Cefiler)

Cefiler is a fully-automated plugin devoted to the identification of cell files provided by light microscopic images. This module allows cellular type (*vessel*, *fiber*, *parenchyma*, ...) to be defined and the geometry of each cell (wall or/and lumen area, wall thickness, circularity,...) to be characterized. The numerical results are saved in csv file.

Important note

This plug-in is in preparation and will be finalized in the coming year.

The Cefiler plug-in is opened from the second icon in the tools bar of Toaster

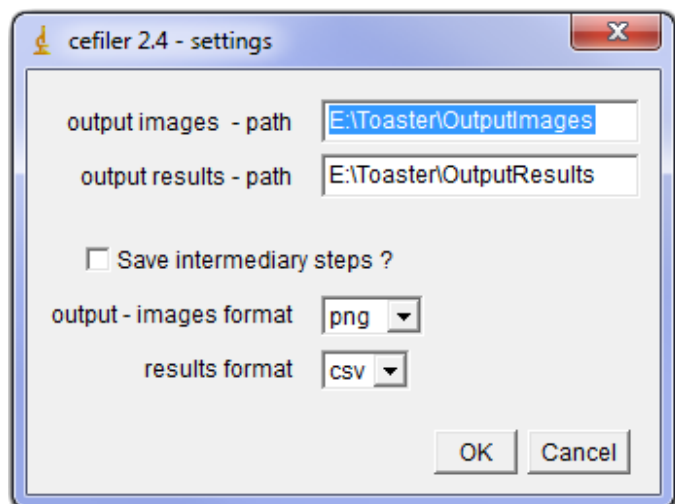


Note

- At least one image must be loaded before to open Cefiler plug-in .

5.1. Cefiler settings

The following dialog box allows specifying the working folders and the export formats.





*The managing aspects are set from the Preferences panel of the Toaster plug-in (see §2).
Are editable the following fields:*

Output images - path: extend name of the folder where will be achieved the output images.

This field is set from the Toaster settings. These images are built to avoid a visual checking after data treatment. The leaves are colored in regard to their position in the treated image: the hue depends on the line, and the saturation on the row in the line (§7).

Output results - path: extend name of the folder where will be achieved the output results.

This field is set from the Toaster settings. These files contain the numerical values of calculated parameters, sorted by natural reading order of leaves – from left to right, from up to down.

Output images format: give the format of the output images

Output results format: give the format of the output numerical files

The csv files are loadable by the usual spreadsheet; the txt files can be imported in the usual spreadsheet.

5.2. Cefiler running

The Cefiler plug-in is ran when you validate your settings: click on button “ok”.

5.3. Example

Data and results

The input data are three jpeg images located into the folder BFile



4000gr10.2.tif



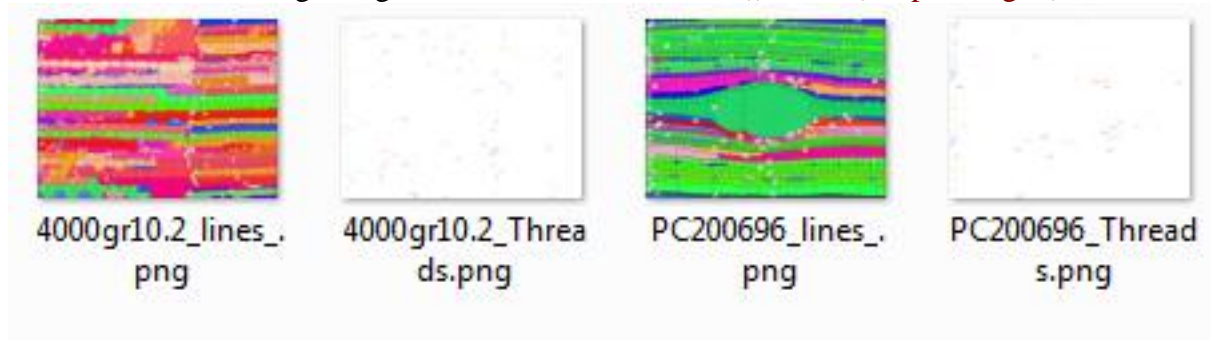
PC200696.JPG



NB: here, the images are produced with the same magnification.

The settings of the §5.1 give the following output data:

- ✓ the checking images achieved in the folder E:\\Toaster\\OutputImages\\BFiles



- ✓ two csv files respectively named *4000gr10.2* and *PC2000696*, achieved in the folder E:\\Toaster\\OutputResults\\BFiles

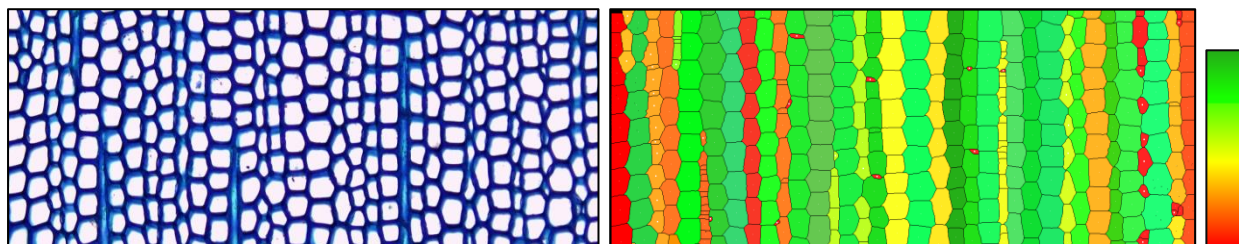
	A	B	C	D	E	F	G	H
1	file	cell	xcenter	ycenter	lumen_aera	ratio_lumer	circularity	Nb Cell
2		0	358	183.0	0	87	0.690215992	6
3		0	372	17.0	0	89	0.792926868	6
4		0	359	31.0	7	116	0.772605028	6
5		0	353	47.0	87	191	0.903442707	6
6		0	340	70.0	20	250	0.589888424	6
7		0	313	86.0	17	192	0.849646217	6
8		36	526	605.0	0	155	0.575357665	1
9		37	1019	510.0	0	146	0.742807744	7
10		37	1013	530.0	0	252	0.533938725	7
11		37	1011	551.0	0	257	0.419514122	7
12		37	1009	570.0	0	152	0.631154279	7
13		37	969	595.0	0	394	0.441631207	7
14		37	939	631.0	16	698	0.769483408	7
15		37	942	658.0	28	268	0.755141322	7
16		40	1018	479.0	0	153	0.646665199	3

Here, the results are given in points. Each file is described by a set of cells.



Focus on visual results (soon implemented)

Reliability coefficients are introduced in the image processing step to qualify the cell organization identification. The reliability coefficients will be essentially used to filter the input data of the statistical studies lead by the botanist scientists or to call attention to special biological configurations.



Here, weakly reliable files appear in red tones as strongly reliable files are in green tones.

5.4. Reference

Brunel, G.; Borianne, P.; Subsol, G.; Jaeger, M., Caraglio, Y., 2013, **Defining reliability coefficients in an automated method of identification and characterization of radial files in microscopic images of gymnosperms** In Nygren, P., Lintunen, A., Godin, C., Nikinmaa, E., Sievänen, R. Eds, Proceedings of the 7th International Conference on Functional Structural Plant Models, Saariselka, Finland, 9-14 June 2013, pp. 82-84

Brunel, G.; Borianne, P.; Subsol, G.; Jaeger, M., 2013, **Simple-graphs fusion in image mosaic. Application to automated cell files identification in wood slices** In Kämäräinen, J. K., Koskela, M. Eds, Image analysis : 18th Scandinavian Conference, SCIA 2013, Espoo, Finland, June 17-20, 2013. Proceedings, 2013, pp. 34-43

Brunel, G.; Borianne, P.; Subsol, G.; Jaeger, M.; Caraglio, Y., 2012 **Automatic characterization of the cell organization in light microscopic images of wood: application to the identification of the cell file** In Guo, Y., Kang, M. Z., Dumont, Y. Eds, Plant growth modeling, simulation, visualization and applications. Proceedings PMA12 : The Fourth International Symposium on Plant Growth Modeling, Simulation, Visualization and Applications, Shanghai, China, 31 October-3 November 2012, pp. 58-65

Kennel, P.; Subsol, G.; Guérout, M.; Borianne, P., 2010, **Automatic identification of cell files in light microscopic images of conifer wood** In Djemal, K., Deriche, M. Eds, 2010 2nd International conference on image processing theory, tools and applications, IPTA 2010, Paris, France, July 7-10, 2010, pp. 93-108